
Article:
Lam, Z., Albaba, S., Study, D.D.D. et al. (1 more author) (2020) Atypical, milder presentation in a child with CC2D2A and KIDINS220 variants. Clinical Dysmorphology, 29 (1). pp. 10-16. ISSN 0962-8827

https://doi.org/10.1097/mcd.0000000000000298

	09/04/19	4 Color Fig(s):0	21:40	Art: CD-D-19-00047

Atypical, milder presentation in a child with *CC2D2A* and *KIDINS220* variants

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With the increasing availability and clinical use of exome and whole-genome sequencing, reverse phenotyping is now becoming common practice in clinical genetics. Here, we report a patient identified through the Wellcome Trust Deciphering Developmental Disorders study who has homozygous pathogenic variants in *CC2D2A* and a de-novo heterozygous pathogenic variant in *KIDINS220*. He presents with developmental delay, intellectual disability, and oculomotor apraxia. Reverse phenotyping has demonstrated that he likely has a composite phenotype with contributions from both variants. The patient is much more mildly affected than those with Joubert Syndrome or Spastic paraplegia, intellectual disability, nystagmus, and obesity, the conditions associated with *CC2D2A* and *KIDINS220* respectively, and therefore, contributes to the phenotypic variability associated with the two conditions. *Clin Dysmorphol* XXX: 000–000 Copyright © 2019 Wolters Kluwer Health, Inc. All rights reserved.

Clinical Dysmorphology 2019, **XXX**:000–000

Keywords: Joubert syndrome, reverse phenotyping, whole-exome sequencing AQ2

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Received 12 May 2019 **Accepted** 19 August 2019

Introduction

Cilia are hair-like organelles that are involved in a variety of important processes from embryonal development to cell signalling (Reiter and Leroux, 2017). They are found on virtually all cells. Due to their widespread presence, a disruption to their function leads to multisystem abnormalities which are recognised as an expanding group of over 30 conditions collectively known as ciliopathies (Reiter and Leroux, 2017).

The number of causative genes associated with ciliopathies is nearing 200 (Reiter and Leroux, 2017). Coiled-coil and C2 domains-containing protein 2A (CC2D2A) is a protein which has been found to localise at the transition zone of cilia and has been demonstrated to form complexes with other proteins (known to cause ciliopathies) that influence ciliogenesis (Chih *et al.*, 2011; Garcia-Gonzalo *et al.*, 2011) and the movement of proteins between the ciliary and plasma membranes (Chih et al., 2012).

Variants in *CC2D2A* are associated with the autosomal recessive ciliopathy Joubert syndrome (Noor *et al.*, 2008), COACH syndrome (Doherty *et al.*, 2010), and Meckel syndrome (Tallila *et al.*, 2008). Joubert syndrome was first described in 1969 by Marie Joubert (Joubert *et al.*, 1969). It is characterised by the presence of the molar-tooth sign (MTS) on brain imaging due to abnormal development of the cerebellar vermis and brainstem, hypotonia, and developmental delay (Maria *et al.*, 1999). The diagnosis is, therefore, made on the grounds of imaging and clinical examination.

We are discovering that Mendelian disorders with well-defined phenotypes can be more variable and milder than originally described. This may be partly due to the fact that initial studies focused on severe developmental delay phenotypes but with wider access to next-generation sequencing, patients with non-distinctive, milder phenotypes are having more advanced genetic testing. Joubert syndrome and its associated genes are no exception (Irfanullah *et al.*, 2016; Méjécase *et al.*, 2019).

Here, we report a patient identified through the Deciphering Developmental Disorders (DDD) study (Wright *et al.*, 2015) who is homozygous for a pathogenic variant in *CC2D2A* and heterozygous for a previously unreported pathogenic variant in *KIDINS220*. We provide a comprehensive review of the *CC2D2A* variants that have been published so far to add to the work previously published by Bachmann-Gagescu *et al.* (2012).

Materials and methods

The patient was referred to a UK clinical genetics centre and subsequently recruited to the DDD study. Trio-based AQ3 exome sequencing was performed followed by analysis as per the DDD protocol described previously (Wright *et al.*, 2015). Variants identified by the DDD study were validated using accredited UK NHS diagnostic genetics laboratories prior to informing patients. Informed consent was obtained from the patient's parents for inclusion in this report.

DOI: 10.1097/MCD.0000000000000298

<zdoi;10.1097/MCD.0000000000000298>

09/04/19 | 4 Color Fig(s):0 | 21:40 | Art: CD-D-19-00047

AQ4 The DECIPHER ~~database of genomic variation~~ was searched for patients recruited to the DDD study who were found to have variants in *CC2D2A* (~~DECIPHER~~).

A GeneMatcher request was created to identify other patients with Joubert Syndrome and *CC2D2A* variants (Sobreira *et al.*, 2015).

Results

The DECIPHER database search identified a total of four patients from the DDD study with variants in *CC2D2A*. The pathogenicity of the variants was classified as uncertain in two patients, and one variant was not allocated a pathogenicity classification.

The GeneMatcher request did not identify any additional patients.

We, therefore, describe one patient who is homozygous for GRCh37(hg17) NM_001080522.2 c.2671G>A, p.(Glu891Lys) missense variant in the *CC2D2A* gene (~~Decipher~~ ID 293170). This variant is biparentally inherited and has been previously reported in a compound heterozygous patient (Bachmann-Gagescu *et al.*, 2015). A de-novo heterozygous nonsense variant in the *KIDINS220* gene was also found; GRCh37(hg17) NM_020738.3 c.1369C>T, p(Gln457Ter). This truncating variant is likely to be pathogenic and has not been previously reported in the literature.

Clinical report

Our patient is a male born to consanguineous parents. He has a brother and two cousins who have been diagnosed with autism spectrum disorder. The pregnancy was uncomplicated. He was delivered at 38 weeks gestation weighing 2.77 kg (11th centile). There were no immediate complications after delivery. He is developmentally delayed; he walked after the age of two and had only one to two words at the age of three and a half.

At the age of six and a half, when he was assessed by the clinical genetics team, he was in mainstream school with one-to-one support. He had been diagnosed with autism spectrum disorder. There were no reports of seizure activity. Parents mentioned that tantrums were a problem, and he is particular with food. On examination, his height was 119 cm (53rd centile), weight was 21 kg (36th centile), and he had an occipital frontal circumference of 52 cm (57th centile). He was not dysmorphic. He had poor horizontal saccadic eye movements which he has had since infancy and a typical head thrust associated with this. An MRI scan of the brain and ultrasound scans of the liver and kidneys were unremarkable. The MTS was absent.

Published variants

CC2D2A variants, with and without available phenotypic information, published after the work by Bachmann-Gagescu and colleagues can be found in Tables 1 and 2, T1 respectively. Variants that have been described in a heterozygous state can be found in Table 3. T3

In Table 2, Ben-Salem *et al.*, (2014) identified a novel variant c.4258G>A, (p.Arg1528His), however, following a review of the variants we noted that the nucleotide at position 4258 is cytosine. We, therefore, believe that this variant should be c.4583G>A which would then result in p.Arg1528His. The senior author in this paper ~~has been~~ contacted to clarify this variant. T2

Discussion

A number of *CC2D2A* variants have been described in the literature since the last summary a few years ago (Bachmann-Gagescu *et al.*, 2012) (see Tables 1–3). The majority of variants have been identified in a compound heterozygous state in individuals who have a diagnosis of Joubert syndrome or Meckel syndrome. We would like to highlight from these summary tables an interesting variant that was described in an individual with Meckel syndrome. Meckel syndrome is a *CC2D2A* allelic disorder with a more severe phenotype. In general, missense variants lead to Joubert syndrome and null alleles lead to MKS (Mougou-Zerelli *et al.*, 2009). Takenouchi *et al.* (2017) described a transposable element insertion in *CC2D2A*; a novel mutation type associated with ciliopathies. This along with a maternally inherited frameshift variant was thought to be the cause of Meckel syndrome in this individual. Transposable element insertions have also been described in Alström syndrome and Bardet Biedl Syndrome (Taşkesen *et al.*, 2011; Tavares *et al.*, 2018) suggesting that this type of variant may be important to bear in mind for cases where only one variant has been identified with the usual analytical pipelines.

Joubert syndrome is genetically heterogeneous and phenotypically very variable. Genotype-phenotype correlations exist which is helpful in informing patients and clinicians about the potential development of complications in the future and guiding monitoring (Bachmann-Gagescu *et al.*, 2015). Joubert syndrome patients with *CC2D2A* variants seem to have much lower chances of developing organ-specific complications compared to other genes such as *TMEM67* where patients are highly likely to have colobomas and develop liver disease (Bachmann-Gagescu *et al.*, 2012). From an ophthalmological perspective, *CC2D2A* has been shown to be associated with oculomotor apraxia in almost all cases with a high proportion also having nystagmus (Brooks *et al.*, 2018). There have been very few reports of retinal disease in *CC2D2A* patients with only one patient in two series of patients being identified (Bachmann-Gagescu *et al.*, 2015; Brooks *et al.*, 2018).

There is recent evidence to suggest that the phenotype associated with *CC2D2A* variants could be milder than classic Joubert syndrome. From the literature search, three brothers from a consanguineous family have been

Table 1 **Case reports with phenotypical information available in the NM_001080522.2 *CC2D2A* gene**

Reference	Identification	Allele 1	Allele 2	Diagnosis	MTS	ENC	DD/ID	OA/N	RCD	Renal	Liver	PD	Other
Srour *et al.*, 2012b	1610.572	c.2181+1G>A	c.4667A>T p.(Asp1556Val)	JS	+	?	+	+	–	–	–	–	Hypotonia, ataxia
Xiao *et al.*, 2017	Proband	c.2848C>T, p.(Arg950*)	c.2581G>A p.(Asp861Asn)	JS	+	?	+	+	?	?	?	?	Hypotonia
Bachmann-Gagescu *et al.*, 2015	UW262-3	c.3347C>T, p.(Thr1116Met)	c.4741A>G p.(Thr1581Ala)	JS	+	?	?	+	–	–	–	–	
Srour *et al.*, 2012a	1295.473	c.3376G>A, p.(Glu1126Lys)	c.4667A>T p.(Asp1556Val)	JS	+	?	+	+	–	–	–	–	Head titubation, Hypotonia, ataxia
Srour *et al.*, 2012a	1332.484	c.3376G>A, p.(Glu1126Lys)	c.4667A>T p.(Asp1556Val)	JS	+	?	+	+	–	–	–	–	Hypotonia, ataxia
Srour *et al.*, 2015	1342.488	c.3376G>A, p.(Glu1126Lys)	c.4667A>T p.(Asp1556Val)	JS	+	?	+	+	–	–	–	–	Hypotonia, ataxia, autism, ADHD
Srour *et al.*, 2015	1343.488	c.3376G>A, p.(Glu1126Lys)	c.4667A>T p.(Asp1556Val)	JS	+	?	+	+	–	–	?	–	Hypotonia, ataxia
Srour *et al.*, 2012b	1356.492	c.3450_3452del, p.(Val1151del)	c.4667A>T p.(Asp1556Val)	JS	+	?	+	+	-	-	-	-	Hypotonia, ataxiaoromotor apraxia, dysphasia
Vilboux *et al.*, 2017	39-31-483	c.3452T>C, p.(Val1151Ala)	c.248-4_248-3insAAGTTTT	JS	+	?	?	–	–	–	–	–	
Syzmanska et al., 2012	158	c.3540delA, p.(Arg1180Serfs*7)	c.3540delA, p.(Arg1180Serfs*7)	MKS/MKS-like	?	+	?	?	?	PC	+	+	Cleft lip/palate + other malformations, see paper for further details
Syzmanska et al., 2012	180	c.3540delA, p.(Arg1180Serfs*7)	c.3540delA, p.(Arg1180Serfs*7)	MKS/MKS-like	?	+	?	?	?	PC	–	+	Dandy-Walker malformation
Bachmann-Gagescu *et al.*, 2015	UW088-3	c.3989G>A, p.(Arg1330Gln)	c.3743_3746dupTGGT p.(Pro1250Glyfs*11)	JS	+	?	+	+	–	–	–	–	
Bachmann-Gagescu *et al.*, 2015	UW088-4	c.3989G>A, p.(Arg1330Gln)	c.3743_3746dupTGGT p.(Pro1250Glyfs*11)	JS	+	?	+	+	–	–	–	–	
Al-Hamed *et al.*, 2016	FT-26	c.4437+1G>A	c.4437+1G>A	?	?	+	?	?	?	PC	?	?	Ascites
Incecik et al., 2012	1	c.4452C>T, p.(Arg1518Trp)	c.4452C>T, p.(Arg1518Trp)	JS	+	?	+	+	?	–	–	+	Episodic hyperpnoea, coloboma, hypotonia, cerebellar vermis aplasia, vermian cleft
Incecik et al., 2012	10	c.4452C>T, p.(Arg1518Trp)	c.4452C>T, p.(Arg1518Trp)	JS	+	?	+	+	?	–	–	?	Episodic hyperpnoea, ataxia, hypotonia, cerebellar vermis aplasia, vermian cleft
Jones *et al.*, 2014	Proband	c.4550C>G, p.(Thr1517Ser)	c.3774dupT p.(Glu1259*)	MKS	?	+	?	?	?	PC	?	–	
Shaheen *et al.*, 2013	MKS _F8	c.4531T>C, p.(Trp1511Arg)	c.4531T>C p.(Trp1511Arg)	MKS	?	+	?	?	?	PC	?	+	See paper for further details
Shaheen *et al.*, 2013	MKS _F14	c.4531T>C, p.(Trp1511Arg)	c.4531T>C p.(Trp1511Arg)	MKS	?	+	?	?	?	PC	?	+	See paper for further details
Srour *et al.*, 2012b	994.385	c.4559A>G, p.(Asn1520Ser)	c.3376G>A p.(Glu1126Lys)	JS	+	?	+	+	–	–	–	–	
Srour *et al.*, 2012b	1303.385	c.4559A>G, p.(Asn1520Ser)	c.3376G>A p.(Glu1126Lys)	JS	–	?	–	+	–	–	–	–	
Kroes *et al.*, 2016	1-34	c.4577C>A, p.(Thr1526Asn)	c.3289delG, p.(Val1097Phefs*2)	JS	+	?	+	+	?	RI	–	?	
Bachmann-Gagescu *et al.*, 2015	UW287-3	c.4600T>G, p.(Leu1534Val)	c.1017+1G>A	JS	+	?	?	+	+	–	+	–	
Srour *et al.*, 2012b	1223.447	c.4667A>T, p.(Asp1556Val)	c.3376G>A p.(Glu1126Lys)	JS	+	?	+	+	–	–	–	–	Oromotor apraxia

Table 1 ***(continued)***

Reference	Identification	Allele 1	Allele 2	Diagnosis	MTS	ENC	DD/ID	OA/N	RCD	Renal	Liver	PD	Other
Srour *et al.*, 2012b	978.379	c.4702T>C, p.(Tyr1568His)	c.3376G>A, p.(Glu1126Lys)	JS	+	?	+	+	–	–	–	–	Resp abnormalities, hypotonia, ataxia, epilepsy, oromotor apraxia
Méjécase *et al.*, 2019	CIC02583	c.4730_4731delinsTGTATA p.(Ala1577Valfs*5)	c.2774G>C p.(Arg925Pro)	Isolated RCD	–	?	?	+	+	+	?	?	HTN, haematuria, nephrotic-range proteinuria. Normal MRI. Homo CNGA3 variant. Compound heterozygous CUBN variants
Méjécase *et al.*, 2019	CIC02584	c.4730_4731delinsTGTATA p.(Ala1577Valfs*5)	c.2774G>C p.(Arg925Pro)	Isolated RCD	–	?	?	?	+	–	?	?	Normal renal function and brain structure. Het CNGA3 variant
Méjécase *et al.*, 2019	CIC02585	c.4730_4731delinsTGTATA p.(Ala1577Valfs*5)	c.2774G>C p.(Arg925Pro)	Isolated RCD	–	?	?	?	+	+	?	?	Haematuria, proteinuria. Het CNGA3 variant. Compound heterozygous CUBN variants
Takenouchi *et al.*, 2017	Proband	exonic transposable element insertion in exon 7	c.4582_4583delCG p.(Arg1528Serfs*17)	MKS	?	+	+	?	?	PC	–	+	hydrocephalus, VP shunt, apnoeic spells, poor growth

DD/ID, developmental delay/intellectual disability; ENC, encephalocoele; JS, Joubert syndrome; JSRD, Joubert syndrome-related disorder; MKS, Meckel syndrome; MTS, molar-tooth sign; NPHP, nephronophthisis; OA/N, oculomotor apraxia/nystagmus; PC, polycystic; PD, polydactyly; RCD, rod-cone dystrophy; RI, renal insufficiency; ?, no information available; +, present; –, absent; ~~##, variant may be in cis or trans.~~

described. They underwent whole-exome sequencing for rod-cone dystrophy and were found to be compound heterozygous for variants in *CC2D2A* (Méjécase *et al.*, 2019). Similar to our patient, they all have normal brain imaging with absence of the MTS. Our patient, therefore, provides further support of a milder phenotype associated with *CC2D2A*; he presents with normal imaging of the brain, kidneys, and liver but with oculomotor apraxia and a homozygous variant in *CC2D2A* which has been previously reported in a compound heterozygous state in a patient with classic Joubert syndrome (Bachmann-Gagescu *et al.*, 2015). There is, unfortunately, no phenotypic information available about the latter patient other than a diagnosis of Joubert syndrome (see Table 2). Nearly 14 000 children and their parents were recruited to the DDD study, however, only two patients with variants in *CC2D2A* were identified (~~DECIPHER~~); one of which is not thought to be significant in terms of molecular finding and phenotypic fit. This may suggest that either patients with *CC2D2A* variants present with a recognisable phenotype, such as Joubert syndrome, and therefore were not recruited to the study, or the phenotype is mild and therefore the patients did not meet eligibility criteria for recruitment. With the increasing availability of next-generation sequencing, those with milder phenotypes will have genetic testing and the latter group of patients may come to light following reverse phenotyping.

There may be several reasons as to why these patients are less severely affected than one would normally expect with biallelic variants in other Joubert syndrome genes. The first reason may just be the phenotypic variability associated with the gene which is becoming more apparent with reverse phenotyping. This has been described in other ciliopathy genes such as *INPP5E* (de Goede *et al.*, 2016). *INPP5E* variants usually lead to Joubert syndrome, however, de Goede *et al.* (2016) describe two branches of a consanguineous family who presented with developmental delay and learning difficulties for assessment. The family phenotype was variable but the authors concluded that all were within the spectrum of INPP5E-related ciliopathies.

The second is the possibility that modifier genes play a part in the more subtle phenotypes of these families, however, further investigations will be required to determine this possibility in our case. The intricacies surrounding ciliopathy phenotypes and variation have been previously well described (Reiter and Leroux, 2017).

Our patient was also found to be heterozygous for a likely pathogenic variant in *KIDINS220* (Kinase D-interacting substrate of 220 kDa). The protein was first identified nearly 20 years ago (Iglesias *et al.*, 2000) as a substrate of protein kinase D which has been found to be important in the development of the neural and cardiovascular systems (Cesca *et al.*, 2011; Cesca *et al.*, 2012).

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Table 2 Case reports with no phenotype information in the NM_001080522.2 *CC2D2A* gene

Reference	Identification	Allele 1	Allele 2	Diagnosis
Ben-Salem *et al.*, 2014	MTI-127	c.1412delG, p.(His471Leufs*40)	c.4258G>A[a], p.Arg1528His	JS or JSRD
Bachmann-Gagescu *et al.*, 2015	UW271-3	c.1503_1505delAGA, p.(Lys501_Asp502delinsAsn)	c.2624C>A, p.(Ser875*)	JS
Bachmann-Gagescu *et al.*, 2015	UW301-3	c.2671G>A, p.(Glu891Lys)	c.4843_4846delTCTC, p.(Ser1615Leufs*16)	JS
Watson *et al.*, 2016	4	c.2875del, p.(Glu959Asnfs*3)	c.2875del, p.(Glu959Asnfs*3)	JS/MKS
Bachmann-Gagescu *et al.*, 2015	UW265-3	c.2999A>T, p.(Glu1000Val)	c.2999A>T, p.(Glu1000Val)	JS
Bachmann-Gagescu *et al.*, 2015	UW265-4	c.2999A>T, p.(Glu1000Val)	c.2999A>T, p.(Glu1000Val)	JS
Bachmann-Gagescu *et al.*, 2015	UW320-3	c.3594+5G>A	c.1558C>T, p.(Arg520*)	JS
Watson *et al.*, 2016	3	c.3774dup, p.(Glu1259*)	c.2803C>T, p.(Arg935*)	JS/MKS
Bachmann-Gagescu *et al.*, 2015	UW308-3	c.4226T>C, p.(Ile1409Thr)	c.4226T>C, p.(Ile1409Thr)	JS
Bachmann-Gagescu *et al.*, 2015	UW307-3	c.4491A>C, p.(Gln1497His)	c.3596T>C, p.(Ile1199Thr)	JS

JS, Joubert syndrome; JSRD, Joubert syndrome-related disorder; MKS, Meckel syndrome.
[a]Please see the results section regarding a discrepancy for this variant.

Table 3 Case reports with a single heterozygous variants in the NM_001080522.2 *CC2D2A* gene

Reference	Identification	Allele 1	Allele 2	Diagnosis	MTS	ENC	DD/ID	OA/N	RCD	Renal	Liver	PD	Other
Kroes *et al.*, 2016	1–37	Not identified	c.949G>A p.(Gly317Arg)	JS	?	?	?	?	?	?	?	?	B9D1 2 variants
Kang *et al.*, 2016	11	Not identified	c.4202C>G p.(Thr1401Ser)	NPHP-related ciliopathy	?	?	?	?	?	RI	+	?	
Kroes *et al.*, 2016	2–47	Not identified	c.4553G>A p.(Arg1518Gln)	JS	?	?	?	?	?	?	?	?	Het for C5orF42 variants
Méjécase *et al.*, 2019	Méjécase *et al* 2019	c.3182+355_3825del[a]	c.2774G>C p.(Arg925Pro)	Simplex RCD					+				

DD/ID, developmental delay/intellectual disability; ENC, encephalocoele; JS, Joubert syndrome; JSRD, Joubert syndrome-related disorder; MKS, Meckel syndrome; MTS, molar-tooth sign; NPHP, nephronophthisis; OA/N, oculomotor apraxia/nystagmus; PC, polycystic; PD, polydactyly; RCD, rod-cone dystrophy; RI, renal insufficiency; ?, no information available; +, present; –, absent.
[a]Variant may be in cis or trans.

Heterozygous truncating variants in *KIDINS220* have been associated with spastic paraplegia, intellectual disability, nystagmus, and obesity (SINO – OMIM 617296) (Josifova *et al.*, 2016; Yang *et al.*, 2018). Pathogenic homozygous variants seem to be associated with a more severe phenotype with limb contractures and hydrocephalus (Mero *et al.*, 2017).

In comparison to the other cases described in the literature, our patient is phenotypically milder. The three cases reported by Josifova *et al.* (2016) were dysmorphic with abnormal brain MRI. All had spastic paraplegia and had weights over the 80th centile; our patient had none of these features. Due to the small number of published cases, it is difficult to know whether our patient expands the phenotype associated with variants in *KIDINS220* or whether this is a reflection of patient ascertainment bias; two out of the three patients described by Josifova *et al.* (2016) were identified from their phenotype before having a large 'obesitome' gene panel which included ~~KIDINS220.~~

Due to the very few patients reported with pathogenic variants within the *KIDINS220* gene and the limited functional work performed, the associated pathogenic mechanism of disease remains unclear. One might conclude from the current reports in the literature that complete loss of KIDINS220 expression leads to a severe phenotype, while monoallelic expression of this protein leads to a mild phenotype, such as in our case. However, this conclusion is not supported by population data. In addition, the reported truncating variants associated with possible expression of a shorter protein could support a gain of function mechanism in this gene (Josifova *et al.*, 2016; Mero *et al.*, 2017; Yang *et al.*, 2018), again however, this is also not supported by population data in gnomAD. When examining this data (data last accessed 20 February 2019), three heterozygote truncating variants were identified within the last exon or the 50 nucleotides in the penultimate exon, predicting the translation of a shortened protein. Also, 12 heterozygote truncating variants were seen which are predicted to result in protein haploinsufficiency. The *KIDINS220* gene has a low probability of LoF intolerance (PLI of 0.03). Therefore, we alternatively propose that incomplete penetrance is likely to be associated with pathogenic variants in this gene. We do not recommend the use of PVS1 (ACMG classifier) in the interpretation of LoF variants within this gene but to instead consider the other available evidence in a case by case basis. In our patient, the *KIDINS220* c.1369C>T, p.(Gln457Ter) was *de novo* (PS2 strong) and it was not present in control populations in gnomAD (PM2_moderate), giving a likely pathogenic classification. AQ6

In summary, we describe a 7-year old boy, who is an example of the ever growing cohort of patients who are being 'reversed phenotyped,' who present with an atypical phenotype with pathogenic variants in *CC2D2A* and *KIDINS220*. Further phenotype-genotype studies in these two genes may give further information regarding their associated phenotypic spectrums.

Acknowledgements

This study makes use of data generated by the DECIPHER community. A full list of centres who contributed to the generation of the data is available from http://decipher.sanger.ac.uk and via email from decipher@sanger.ac.uk. Funding for the project was provided by the Wellcome Trust. The DDD study presents independent research commissioned by the Health Innovation Challenge Fund [grant number HICF-1009-003] see Nature 25533962 or www.ddduk.org/access.html for full acknowledgement.

Conflicts of interest

AQ7 There~~re are~~ no conflicts of interest.

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